New and Notable

Modeling Mitochondrial ROS: A Great Balancing Act

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> "So be sure when you step, Step with care and great tact. And remember that life's A Great Balancing Act. Just never forget to be dexterous and deft, and never mix up your right foot with your left."
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> —Dr. Seuss, *Oh, The Places You'll Go!* Random House, 1990.

The benefits and risks of a cell's use of O_2 can be a tricky balance. Consumption of O_2 by mitochondria greatly enhances the efficiency of aerobic metabolism, allowing generation of 15-fold more ATP than is possible by anaerobic glycolysis alone. Most O_2 consumption occurs in the electron transport chain. Under normal conditions, the electron transport chain takes high-energy electrons from NADH or $FADH_2$ to generate a proton concentration gradient and membrane potential gradient between the inner mitochondrial space and the mitochondrial matrix. O_2 plays a key role by capturing four low energy electrons coming out of the electron transport chain, forming H_2O in the process. The proton gradient is used to power ATP synthase, which harnesses the energy from protons flowing back into the mitochondrial matrix to phosphorylate ADP, yielding ATP. Yet if a high-energy electron leaks from the electron transport chain too early, it may be captured by O_2 to form the reactive oxygen species (ROS) known as superoxide (O_{2-}). Superoxide can be quite damaging because it has an extremely high affinity for electrons, ripping them away from nearby proteins, lipids, and nucleic acids via oxidation. Indeed, ROS species play a key role in a wide range of pathologies such as atherosclerosis (1), Alzheimer's disease (2), and cancer (3). Therefore the cell needs to balance the benefits of an efficient aerobic metabolism with the risks of generating toxic ROS.

Although the interplay between ATP synthesis and ROS generation has been studied intensively, there have emerged apparent inconsistencies between explanations of the conditions and mechanisms responsible for mitochondrial ROS production from isolated mitochondria and intact cells. As an attempt to resolve these discrepancies, Aon et al. (4) showed that ROS overflow is minimal at intermediate redox states, increasing in either highly reduced/high mitochondrial membrane potential environments (e.g., high workload) or highly oxidized environments (e.g., hypoxia). ROS increased in highly reduced environments due to enhanced ROS production, whereas ROS increased in highly oxidative environments due to lower ROS scavenging. Thus increased mitochondrial ROS arises from imbalance of ROS production and ROS scavenging. Based on this data, Aon et al. (4) proposed the Redox-Optimized ROS Balance hypothesis, that mitochondria have evolved an optimal, intermediate redox state to maximize energy output while minimizing ROS overflow.

In this issue of the *Biophysical Journal*, Gauthier et al. (5) develop a mechanistic computational model to better understand the mechanisms underlying this complex balance between mitochondrial ROS production and ROS scavenging. The model was carefully validated against a range of independent experimental data including how ROS production changes as a function of NAD^+/NADH redox potential, mitochondrial matrix pH, substrate, respiratory state, and inhibition of complex I or III. Gauthier et al. (5) do a commendable job of describing conditions where model predictions deviate from experimental data, pointing toward contemporary gaps where mechanisms remain to be identified. During conditions of reverse electron transport, ROS production from complex I has been proposed to occur either at the flavin mononucleotide site (6) or the quinone binding site (7). Model variants based on either of these two potential mechanisms were able to accurately predict ROS production with varying NAD^+/NADH redox potential (5), indicating that further conditions for discriminating these mechanisms are still required. By integrating the electron transport chain model with a minimal model of ROS scavenging, the authors showed how large shifts in redox environment in either direction (toward oxidation or reduction) increase ROS levels (see Fig. 1). Thus this model provides a set of biophysical mechanisms that are sufficient to predict the main features of the Redox-Optimized ROS Balance hypothesis and related experimental data.

So what are the next steps? Gauthier et al. (5) make a number of interesting testable predictions that warrant new experiments, including the relative role of mitochondrial membrane potential and NADH redox state on ROS production and scavenging, respectively. After further validation, this model would be a highly useful new, to our knowledge, module in more comprehensive models of mitochondrial metabolism or multiscale models connecting mitochondrial ROS to cardiac electrophysiology. Such a model would allow prediction of multiscale feedbacks between molecular mechanisms of ROS production/scavenging, calcium dynamics, and ventricular arrhythmia. For example, Christensen et al. (8) have already modeled how oxidation of calcium-calmodulin dependent protein

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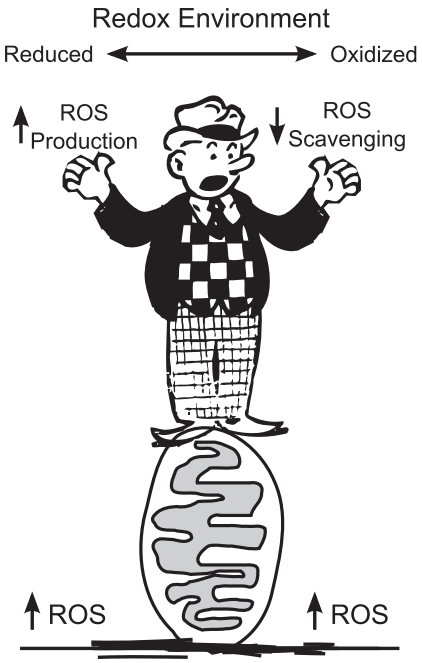

FIGURE 1 Balance of ROS production and ROS scavenging depends on the mitochondrial redox environment.

kinase II prolonged the refractory period of the action potential, increasing susceptibility to conduction block. Zhou et al. (9) showed how ROS-induced ROS release can synchronize mitochondrial depolarization across the entire cell or even propagate to ventricular fibrillation (10). Models of bidirectional links between mitochondrial ROS and signaling networks involved in apoptosis and cardiac hypertrophy are needed as well (11–13). Ultimately, iterative steps of care (solid experimental validation) and steps of deft (multiscale integration) will allow progressive understanding of the mechanisms and functional consequences of mitochondrial ROS, a challenging but critical balancing act.

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